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TECHNICAL PROGRESS REPORT

to the

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

for

NASA Grant NsG 441-63

Title of Project: Investigations in Space-Related Molecular Biology, Including Considerations of the Molecular Organization of Extraterrestrial Matter.

Period of Project: April 1, 1965 to March 31, 1966

Institution: The University of Chicago

Principal Investigator:

Humberto Fernandez-Moran, M.D., Ph.D.
Professor of Biophysics
Department of Biophysics

N66-13830 (ACCESSION NUMBER)
31 (PAGES)
CR 68844 (NASA CR OR TMX OR AD NUMBER)
1 (THRU)
(CODE)
04 (CATEGORY)
FACILITY FORM 602

GPO PRICE $ _____
CFSTI PRICE(S) $ _____
Hard copy (HC) 2.00
Microfiche (MF) .50
ff 653 July 65

RESEARCH PROPOSAL
for
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

TITLE: Integrated Research and Training in "Space-Molecular Biology" for the period April 1, 1966 through December 31, 1966.

INSTITUTION: The University of Chicago

PRINCIPAL INVESTIGATOR: Humberto Fernandez-Moran, M.D., Ph.D.
Professor of Biophysics
Department of Biophysics

PROJECT ABSTRACT:

This research proposal for the newal of NASA Grant NsG 441-63 describes an integrated research and training program, carried out as part of a comprehensive research program in molecular biology in progress at the new research facility for high resolution electron microscopy. In addition to our plans for the continued organization and construction of the special electron microscope laboratories with clean room facilities, our proposed technical program comprises the following major aspects:

(1) We are planning to participate in the LUSTER Sounding Rocket experiments to sample lunar dust near the earth. LUSTER is a sounding rocket program established to collect materials believed to have a lunar origin from the outer fringe of the earth's atmosphere for laboratory analysis. For this purpose, we have designed and constructed a special high vacuum container for transfer of the sampling surfaces to collect extraterrestrial material for electron microscopic, electron diffraction, and electron microprobe analysis under controlled conditions of minimum contamination. We hope to continue and expand our contributions in design and development work towards this program.

(2)(a) Continuation of correlated electron microscope and electron diffraction studies of certain meteorites (Orgueil carbonaceous chondrite) carried out with Dr. Edward Anders and Dr. Frank W. Fitch of the University of Chicago. (b) Continuation of correlated electron microscopical studies of Pre-Cambrian organized systems.

(3) Comprehensive work in experiments with electron microscopy using high-field superconducting solenoid lenses will be continued and extended. Our proposed program includes the following major instrumentation improvements: (1) superconducting lenses with special current Vernier control circuits and improved pole pieces for high resolution superconducting electron microscopy at liquid helium temperatures, in collaboration with Westinghouse Electric Cryogenics Division; (2) improved liquid helium Dewar for cryogenic electron microscopes; (3) an ADL CRYODYNE-(R) helium liquifier for supplying the cryogenic refrigeration required to cool a superconducting device, in collaboration with A.D. Little Company; (4) an improved Schottky-type electron gun with single crystal pointed filaments.

(4) In addition to this instrumentation work, we intend to continue intensive development work on improvement of preparation techniques. This work will be applied to continue the correlated electron microscopic and biochemical investigations of mitochondria, isolated mitochondrial membranes, and associated enzyme and multienzyme complexes. Similar integrated studies on related membrane derivatives in red blood cells, hemocyanins, photoreceptors, chloroplasts, myelin, and erythrocytes will also be continued.

NASA Grant NsG 441-63

ANNUAL PROGRESS REPORT

Principal Investigator

H. Fernandez-Moran

Contract Number

NsG 441-63

Institution

The University of Chicago

Period of Project

April 1, 1965 to
March 31, 1966

Title of Project

Investigations in Space-Related Molecular Biology, Including Considerations of the Molecular Organization of Extraterrestrial Matter.

Following the program set forth in our research proposal, and as described in the enclosed reports, reprints, and preprints, our efforts during the past year have centered on:

I. Specific Research Program.

A. Continued development work on improvement of preparation techniques and instrumentation for high resolution electron microscopy, including further application of low-temperature methods, and the design of new types of high resolution "cryoelectron microscopes" immersed in liquid helium cryostat, using superconducting electromagnetic lenses and image intensifiers with electronic read-out.

1. The key role played by cell membranes and their derivatives has become increasingly apparent, thus making detailed elucidation of the molecular organization of cell membranes one of the most challenging problems of contemporary biomedical research. The shortcomings of our present preparation techniques are particularly central problems now that modern electron microscopes consistently achieve resolutions of the order of 5 to 10 Å and are thus inherently capable of directly visualizing molecular structures in the size range of key enzymes in nuclear proteins.

2. Another of our major efforts has centered on intensive development and application of improved instrumentation for high resolution electron microscopy. By using the full resolving power of modern microscopes, and with the improved preparation techniques made available, the commonly accepted biological applications of electron microscopy can be considerably extended. High resolution electron microscopy has thus become a valuable analytical tool uniquely suited for direct visualization of macromolecular structures in the dried or native hydrated states. Thus, investigators are

provided with reproducible data under controlled experimental conditions which can profitably supplement the results of parallel biophysical and biochemical investigations.

a. One of the principal areas of our instrumentation work is the comprehensive experimentation and development of electron microscopy using high field superconducting solenoid lenses. Already, the resolving power of the electron microscope has extended the range of direct visualization to structural details of the order of a few Angstroms. Considering the numerous complex instrumental and preparative factors involved, the main steps to be taken for attainment of the ultimate theoretical resolution (about 2 Å) are correction of lens aberrations (mainly spherical and chromatic aberrations), stabilization of the lens excitation current, and accelerating of voltage. It has been demonstrated that operation of superconducting solenoid, short-circuited, or "in the persistent current mode," yields large uniform magnetic fields which are highly homogeneous to better than one part in 10^6 to 10^7 and are highly stable and noise-free under appropriately controlled conditions.

Based on previous work in low-temperature electron microscopy, preliminary experiments have been successfully carried out with a simple electron microscope which can be used for transmission electron microscopy and electron diffraction, using high-field superconducting solenoid lenses in an open-air core, liquid helium Dewar, preferably operating in the persistent current mode. These preliminary experiments demonstrated the exceptional stability and high quality of the images at magnifications of 50 to 100 times. Further experiments have been carried out with various types of electron microscopes using high-field superconducting solenoid lenses and accelerating voltages of 50,000 volts. The results obtained with these experiments are providing essential data for the design of new types of miniaturized electron microscopes immersed in a liquid helium cryostat. The combination of optimized instrumental design parameters operative under conditions of minimized specimen perturbation represents one of the most promising coherent experimental approaches towards attainment of the theoretical resolution limit in direct examination of organic and biological structures. We have been

able to confirm the results and demonstrate the long-term stability of the high resolution images obtained with electron microscopes using superconducting solenoid lenses.

b. By further development of the concepts embodied in our cryo-fixation techniques, it has been possible to design a new type of miniaturized high-resolution electron microscope totally immersed in liquid helium, which makes use of completely stable superconducting lenses, improved single crystal pointed filaments and other distinctive features. These "cryoelectron microscopes," operating at temperatures of 1 to 4 degrees Kelvin, would embody the following significant features: 1) highly stable superconducting electro-magnetic lenses, with ripple-free magnetic fields of a persistent current in the optimum case; 2) operation in ultra-high vacuum and low temperatures resulting in decisive advantages of minimized specimen contamination, specimen damage and thermal noise; 3) optimum conditions for both low voltage (i.e. 10 to 100 kV) and high voltage electron microscopy. In addition, the use of high-efficiency image viewing at optimum low temperatures would make it possible to use high-speed cinematography and stroboscopic recording (e.g. obtained through pulsed T-F emission from pointed filaments) for attainment of high temporal resolution combined with high spatial resolution. In principle, such a cryo-electron microscope will also be an ideal device for controlled application of electron microbeams (50 to 500 Å diameter) of precisely defined intensity and duration for ultraminiaturization, storage of information, and in general for controlled irradiation and manipulation of minimum perturbation. The described combination of optimized instrumental design parameters operative under conditions of minimized specimen perturbation represents one of the most promising coherent experimental approaches towards attainment of the theoretical resolution limit (about 2Å) in direct examination of organic and biological structures. Plans are now underway for building a special vibration-free room for installation of the new "cryoelectron microscope." This facility with its 10-ton floating foundation for elimination of ambient vibrations should make it possible to exploit the unique stability of superconducting lenses operating in the persistent current mode for long-term exposures of the order of minutes to hours, instead of the current 5 to 15 second exposures.

c. Development work has continued on an improved Schottky-type electron gun with single crystal pointed filaments. We have been able to substantially increase the specific intensity of the source and the transverse coherence length by changing the gun configurations and using metals, such as molybdenum, for the cathode and anode shields. As a result of these two improvements, it has been possible for the first time to obtain point resolution of the order of 2.8Å and crystalline lattice resolution of labile components, such as sodium chloride, of the order of 2.8Å. The point resolution and the enhanced contrast have been of great use in a number of studies which have issued from this laboratory.

B. Correlated electron microscopic and biochemical investigations were carried out on mitochondrial membranes, membrane derivatives, hemocyanins, and on associated enzyme and multienzyme complexes.

1. Research of mitochondrial membranes has continued jointly with Dr. David Green and his associates of the University of Wisconsin. Improved preparation techniques were applied to these systematic investigations, in which electron microscopy proved to be essential since the mitochondrial membranes are especially suited for direct examination by negative staining and other preparation procedures.

2. In collaboration with our visiting research fellow, Dr. Ernst F. J. van Bruggen, studies were made on the whole range of representative hemocyanin and apohemocyanin structures from different biological origins. This work is reported in two subsequent papers.

   a. In the first paper "Macromolecular organization of hemocyanins and apohemocyanins as revealed by electron microscopy" comparative high resolution electron microscopic studies of the structural organization of representative hemocyanins and apohemocyanins from Mollusca and Arthropoda are described. Mollusca hemocyanins are cylindrical molecules (diameter about 340Å, height ranging from 140Å to 680Å) built up from 3 to 12 rows of subunits. Arthropoda hemocyanins are built from a cubic monomer (105Å) in various stages of organization which is species dependent. Mollusca hemocyanins are distinctly different from Arthropoda hemocyanins, although they seem to be built from analogous subunits. New structural details observed close to the quaternary levels are discussed in relation

to available biochemical and biophysical data on these highly organized macromolecular complexes.

b. The second paper "Reassociation of Hemocyanins from Subunit Mixtures" describes how the dissociation and reassociation reactions of hemocyanin mixtures were studied by electron microscopy. The experiments were done respectively with a mixture of Helix pomatia and Loligo pealei hemocyanin (both belonging to phylum Mollusca) and with a mixture Helix pomatia (a Mollusc) and Limulus polyphemus (an Arthropod) hemocyanins. After reassociation many of the original molecular structures are observed together with a certain amount of smaller and irregularly aggregated material. The importance of these specific reassociation reactions between hemocyanin subunits from different classes and from different phylums is discussed.

3. In collaboration with Dr. Robert Haselkorn and his associates, studies were begun on ribosomal precursors, RNA polymerase, and related enzymes. This work will be reported in 3 subsequent papers which are now in preparation.

a. The first paper "Methionine Starvation Particles from Escherichia coli" reports on the electron microscopical examination of ribonucleoprotein particles that accumulate in E. coli K12 W6 when starved for methionine. They appear to be heterogeneous, without well-defined substructure. Well-defined particles, probably RNA polymerase, are a major contaminant of the preparations.

b. The second paper "Physical Properties of a DNA dependent RNA Polymerase from E. coli" reports that high resolution electron micrographs of E. coli RNA Polymerase, relatively free of nucleic acid have been obtained. The 18S and 25S species of polymerase appear to be one and two hexagonal discs, respectively. A smaller, four subunit square structure is also observed.

c. The third paper "Electron Microscopic and Biochemical Characterization of Fraction I Protein" reports that high resolution electron micrographs of Fraction I protein from Chinese cabbage leaves have been obtained. The protein, which has ribulose 1, 5-diphosphate carboxylase activity appears to be a cube with an edge of about 120Å. Substructure can be seen in individual particles, consistent with a model having 24 subunits, the number prescribed by the available physical and chemical data.

4. Joint studies are being carried out on the KGDC multienzyme complex with Dr. Lester Reed of the University of Texas. The results of this work will be reported in a publication at a later date.

5. In collaboration with Dr. Jerard Hurwitz of the Albert Einstein College of Medicine, New York, we are carrying out investigations of RNA polymerase crystals. These studies have yielded interesting preliminary results which will be described at a later date.

II. Completion of Organization, Testing, and Operation of the Electron Microscope Laboratories and Adjacent Laboratories (Rooms 203B, 205, 207) for the Proposed Research Program.

A. With funds provided by grant AT (11-1) 1344 from the Atomic Energy Commission, grant NsG 441-63 from the National Aeronautics and Space Administration, by grant USPHS MED RES 943 from the National Institutes of Health, and by funds from The University of Chicago, including Otho Sprague Institute and L. Block funds, the following alterations and additions were made in the Adjacent Laboratories (Rooms 203B, 205, 207):

1. Room 203B (230 sq. ft.)

   a. Preparation of room for storage of specimens, equipment, and laboratory apparatus.

   b. Installation of Harris Cascade Refrigeration Biological Storage Machine which operates at temperatures as low as -120°C,

2. Room 205 (230 sq. ft.)

   a. Installation of Siemens Elmiskop EM-II with accessories.

   b. Installation of darkroom equipment to expedite development of plates taken during experiments in this room.

   c. Preparation of room as site of superconducting experiments.

3. Room 207 (460 sq. ft.)

   a. Installation of X-Ray diffraction unit with Kratky Camera.

   b. Development and installation of 2 vacuum pumping units used to pre-pump photographic plates (at a rate of 912 plates per 2 hours), also with capacity to pre-pump 70mm film and camera. The efficiency of these pumps is such that it reduces working time by several hours. Plates, film, and camera were previously pre-pumped in the microscope itself which involves a much longer time.

4. Clean Room Laboratory E-III

   a. Construction of special laboratory in Room P-III, adjacent to darkroom to house Hitachi Perkin-Elmer microscope and accessories.

   b. Installation of High Performance Hitachi Perkin-Elmer Electron Microscope and accessories, including Double Condenser Lens, Electron Diffraction Chamber, Hot and Cold Stages and Image Intensification System.

III. List of Publications for 1965-1966 (6 copies of each are included with this report and renewal application)

1. H. Fernández-Morán, Biological Systems as Formed by Water. Summation and General Discussion, in Proceedings of the New York Academy of Sciences, October 5-8, 1964.

2. H. Fernández-Morán, Electron Microscopy with High-Field Superconducting Solenoid Lenses, in Proceedings of the National Academy of Sciences, Vol. 53, No. 2, pp. 445-451, February, 1965.

3. H. Fernández-Morán, Application of High-Field Superconducting Solenoid Lenses in Electron Microscopy. Abstract in Science, Vol. 147, p. 665, May 1965.

4. E.F.J. van Bruggen and H. Fernández-Morán, Reassociation of Hemocyanins from Subunit Mixtures, submitted to the J. of Mol. Biol. for publication in 1966.

5. H. Fernández-Morán and E.F.J. van Bruggen and M. Ohtsuki, Macromolecular Organization of Hemocyanins and Apohemocyanins as Revealed by Electron Microscopy, Submitted to the J. of Mol. Biol. for publication in 1966.

6. A.J.E. Colvill, E.F.J. van Bruggen, and H. Fernández-Morán, Physical Properties of a DNA Dependent RNA Polymerase from E. coli, prepared in the Department of Biophysics, The University of Chicago, September, 1965.

7. H. Manor, E.F.J. van Bruggen, H. Fernández-Morán, R. Haselkorn, Methionine Starvation Particles from E. coli, prepared in the Department of Biophysics, The University of Chicago, September 1965.

8. R. Haselkorn, H. Fernández-Morán, F.J. Kieras, and E.F.J. van Bruggen, Electron Microscopic and Biochemical Characterization of Fraction I Protein, prepared in the Department of Biophysics of the University of Chicago, September, 1965.

D. Participation in the "Luster" Sounding Rocket Experiment to Sample Lunar Dust near the Earth. (November, 1965).

Following the invitation of Dr. Maurice Dubin, Chief of Interplanetary Dust, Physics and Astronomy Program, OSSA of NASA, and in close collaboration with Neil Farlow of the NASA Ames Research Center, we are participating in the rocket collections scheduled for mid-November, 1965.

LUSTER is a sounding rocket program established to collect materials believed to have a lunar origin from the outer fringe of the earth's atmosphere for laboratory analysis. The flight in which we are participating has been scheduled for the Leonid shower, which has shown a 33-year intensity cycle, and may show a remarkable increase in the 1965 peak year. After extensive discussion with Mr. Neil Farlow, and following the detailed instructions furnished in the guide prepared by Dr. Maurice Dubin, we have proceeded to prepare the sampling slides for the LUSTER project, trying to conform to the most rigorous requirements for controlled exposure of suitable substrates for subsequent high resolution electron microscopy.

With the experience gained from similar flights, and based on our extensive studies on contamination, carried out in the specially equipped clean room laboratories, we have taken an approach which appears to be ideally suited for the collection under high vacuum conditions. For this purpose, in collaboration with the staff of the Biophysics Department and Central Workshop, we proceeded to design and construct a special high vacuum container for transfer of the sampling surfaces to collect extraterrestrial material for electron microscopic, electron diffraction, and electron microprobe analysis under controlled conditions of minimum contamination. (Figures 1-6). This stainless steel container provided with Viton gaskets and ultrahigh vacuum valve can be loaded with several hundred electron microscope specimen holders of platinum, coated with thin-film surfaces of carbon, silicon monoxide, plastic, and appropriate ultraclean surfaces under rigorously clean conditions. In contrast to previous specimens which were mounted on the module pans of the rocket-launched payload without providing for high vacuum conditions during transport, this container makes it possible to maintain a high vacuum of the order of 10^{-7} to 10^{-8} mm Hg during transport to the clean room area where attachment to the module pans of the rocket-launched payload takes place. The specimens are shipped back after exposure under similar high vacuum conditions for direct examination by electron microscopy, electron diffraction, and related electron optical techniques. As shown in the accompanying photographs, the holders of the special high vacuum container for the transfer of sampling surfaces

can be readily dissembled and dismantled with few manipulations under clean room and even high vacuum conditions. They provide secure attachment with a novel device for implacement of several hundred specimen holders coated with ultra-thin (100-300 Å) carbon, Formvar, silicon oxide, and other types of substrates. In addition to this, larger surfaces of freshly cleaved single-crystal mica, which provide atomically smooth surfaces, plastic slides and related collecting surfaces suitable for electron optical studies are included.

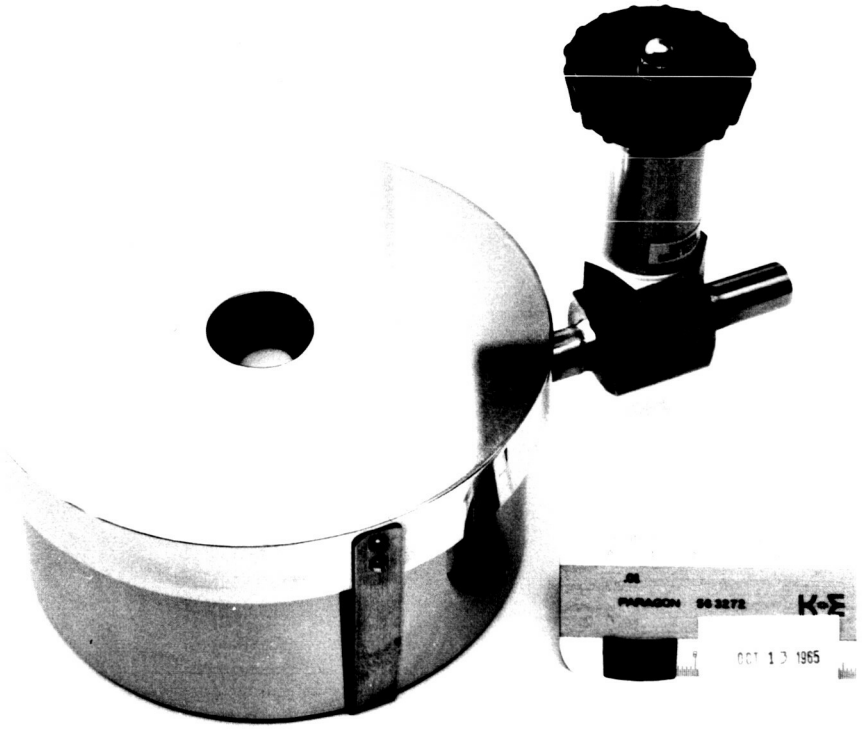
Three identical containers and specimen substrate assemblies were prepared for the LUSTER project micrometeoroid sampling flight scheduled for November, 1965, to provide independent contamination control at the different sites of the experiment: Ames Research Center, the rocket-launching site, and the electron microscope clean room laboratory at the University of Chicago, where the actual payload specimens are to be examined immediately after recovery. These special containers and enclosures were designed and constructed in close collaboration with Mr. L. Ouwerkerk, Mr. John Hanacek, Mr. H. Krebs, Mr. G. Gibson, and Mr. Akerhaugen of the Biophysics Laboratories and workshops in our Research Institutes. It should be noted that the Metal Workshop of the Research Institutes is particularly well-equipped for this type of work and has had considerable experience in similar types of experiments conducted by Dr. John Simpson and his colleagues of the Enrico Fermi Institute for Nuclear Studies here at the University.

Based on the interesting observations reported by C.L. Hemenway and R.K. Soberman in their paper ("Studies of Micrometeorites Obtained from a Recoverable Sounding Rockets, " in the Astronomical Journal, Vol. 67, No. 5. June, 1952, page 256) we have introduced a sampling slide of lucite. In their report, Dr.s Hemenway and Soberman indicate that " micrometeorite particles were found to be sufficiently embedded in the lucite that we were unable to remove them from the substrate." This is an important observation since most of the particles are larger than a few microns and hence impenetrable to the electron beam and inadequte for electron microscopy. It was,therefore, necessary in previous experiments to bombard the micrometeorite particles with an intense electron beam to evaporate the constituent material which recondenses near the particle. In order to avoid this destructive and rather inadequate mode of analysis, we propose to actually prepare serial ultrathin sections (100-200 Å thick) of the particles embedded in the lucite by using the special diamond knife and special ultramicrotome developed by Fernandez-Moran (1953-55).

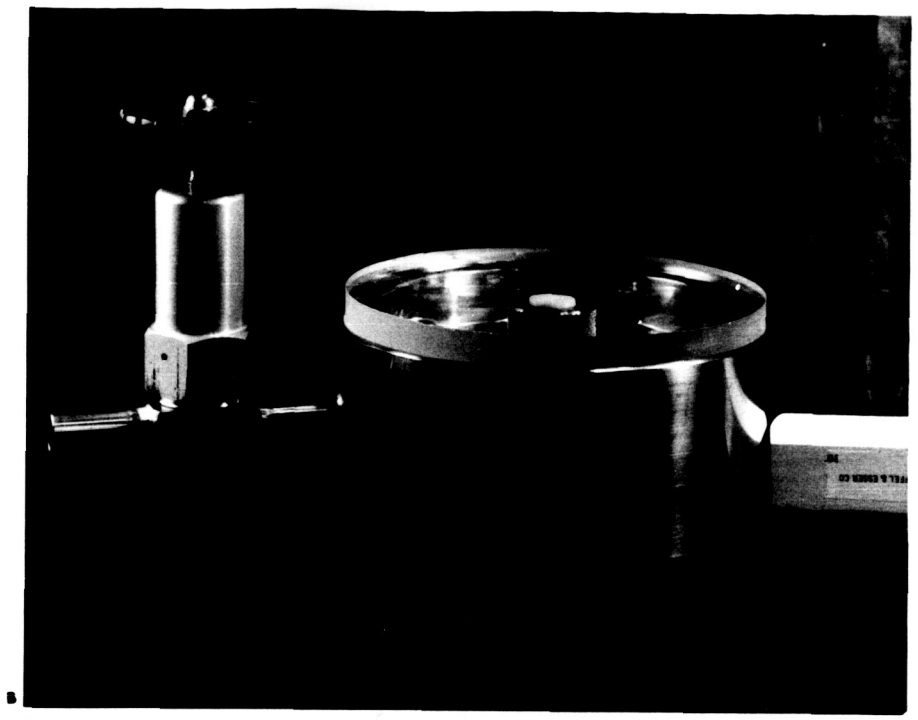
Following our original work on ultrathin sectioning of metals and hard materials, it has been possible during

the past ten years for a number of investigators (e.g.: L. Reimer, " The Thin Section Method in Metalographic Investigation with the Electron Microscope," Z. Metallkunde, 50, 37, 1959 A; Z. Metallkunde, 50, 606, 1959 D; Fernandez-Moran, H., "Applications of a Diamond Knife for Ultrathin Sectioning of the Fine Structure of Biological Tissues and Metals," Journal Biophys. and Biochem. Cytology, 2, Supplement, pg. 29, 1956) to carry out routinely ultrathin sectioning of hard materials and metals which are suitable for high resolution electron microscopy and electron diffraction. We therefore believe that the addition of a lucite slide would make it possible to collect the hard micrometeorite particles in sufficient numbers and under favorable conditions for subsequent examination by ultrathin sectioning using the electron microscope and electron diffraction. If this approach should prove successful it would represent a distinct improvement over the electron bombardment techniques used previously and thus give us more information on the structure and composition of the micrometeorite particles and other extraterrestrial materials.

We have embarked on this project with great interest in collaboration with our colleagues and with a prospective graduate student. It would give us indispensable experience in the sampling and examination of extraterrestrial material in addition to contributing potentially significant information on the composition of lunar dust. In view of the continuing nature of the projected experiments, we anticipate that all of the preparations that have been made here in the clean room laboratories, specially designed for this purpose, will now become of critical importance. In addition, we are requesting continued support in order to make the special ultrahigh vacuum hydrocarbon-free chambers for preparation of thin samples, as well as for the design of special containers and container transfer systems which would make it possible to transfer the specimens from the high vacuum transfer containers to the electron microscope without the possibility of additional contamination. In general, the problem of elimination of contamination is of critical importance in any investigation of this type. We feel that we are particularly well-equipped for this type of work and hope for continued support for these endeavors.



(1) FIG. 1 (a,b): HIGH VACUUM CONTAINER SPECIALLY DESIGNED FOR TRANSFER OF SAMPLING SURFACES TO COLLECT EXTRATERRESTRIAL MATERIAL FOR ELECTRON MICROSCOPIC, ELECTRON DIFFRACTION AND MICROPROBE ANALYSIS UNDER CONTROLLED CONDITIONS OF MINIMUM CONTAMINATION. THIS STAINLESS STEEL CONTAINER PROVIDED WITH VITON GASKETS AND ULTRAHIGH VACUUM VALVE CAN BE LOADED WITH SEVERAL HUNDRED ELECTRON MICROSCOPE SPECIMEN HOLDERS, SINGLE-CRYSTAL MICA, PLASTIC AND OTHER THIN-FILM SURFACES UNDER RIGOROUSLY CLEAN CONDITIONS. IT IS MAINTAINED AT A HIGH VACUUM OF 10^{-7} to 10^{-8} mm Hg DURING TRANSPORT TO THE CLEAN-ROOM AREA WHERE ATTACHMENT TO THE MODULE PANS OF THE ROCKET-LAUNCHED PAYLOAD TAKES PLACE, AND THE SPECIMENS ARE SHIPPED BACK AFTER EXPOSURE UNDER SIMILAR HIGH-VACUUM CONDITIONS FOR DIRECT EXAMINATION BY ELECTRON MICROSCOPY. CONTAINERS AND SPECIMEN HOLDERS WERE DESIGNED, CONSTRUCTED, AND TESTED BY STAFF OF ELECTRON MICROSCOPE FACILITY, BIOPHYSICS DEPARTMENT, AND CENTRAL WORKSHOP OF THE RESEARCH INSTITUTES, UNIVERSITY OF CHICAGO. (Scale in mm.)



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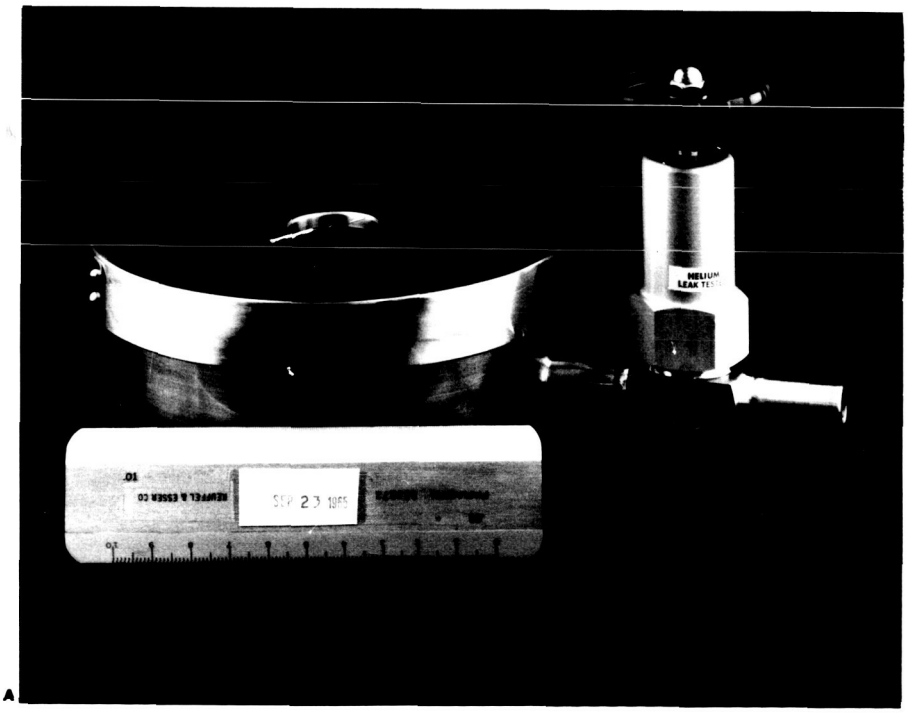

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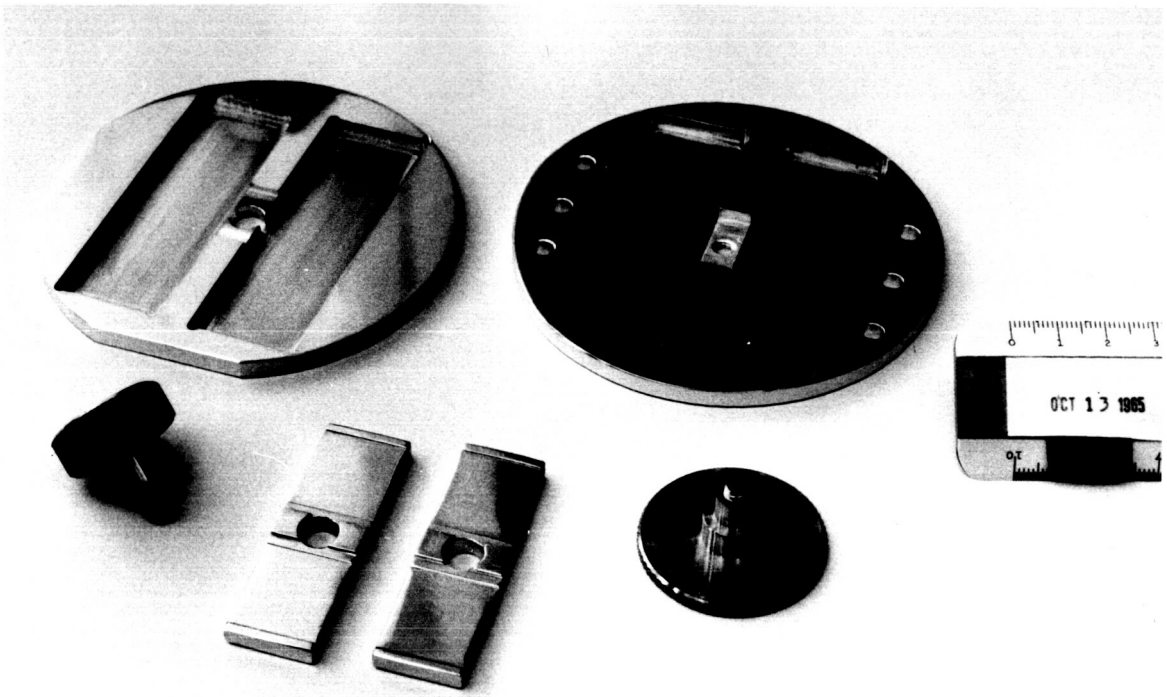
(2)

FIG. 2 (a,b): SPECIAL HIGH-VACUUM CONTAINER FOR TRANSFER OF SAMPLING SURFACES TO COLLECT EXTRATERRESTRIAL MATERIAL FOR ELECTRON MICROSCOPIC, ELECTRON DIFFRACTION AND MICROPROBE ANALYSIS UNDER CONTROLLED CONDITIONS OF MINIMUM CONTAMINATION. STAINLESS STEEL CONTAINER PROVIDED WITH VITON GASKETS, GLASS COVER PLATE, AND ULTRAHIGH VACUUM VALVE CAN BE LOADED WITH BI-METAL HOLDERS FOR ELECTRON MICROSCOPE SPECIMEN HOLDERS OF PLATINUM AND OTHER TYPES COATED WITH THIN-FILM SURFACES OF CARBON, SINGLE-CRYSTAL MICA, AND APPROPRIATE ULTRA-CLEAN SURFACES OF STABLE-CRYSTAL MICA FRESHLY CLEAVED, AND MAINTAINED THROUGHOUT AT HIGH VACUUM (10^{-7} TO 10^{-8} mm Hg) FROM TAKE-OFF TO ULTRA-HIGH VACUUM, ATTACHMENT TO MODULE PANS OF ROCKET-LAUNCHED PAYLOAD, AND RETURN WITH TO ELECTRON MICROSCOPE LABORATORY. THREE IDENTICAL CONTAINERS WERE PREPARED (A "FLIGHT" PLUS SPARE (Nov. 10, 1965) TO PROVIDE INDEPENDENT CONTAMINATION CONTROLS AT DIFFERENT SITES OF EXPERIMENT, TO BE CARRIED OUT AT ELECTRON MICROSCOPY LABS., BIOPHYSICS DEPT., UNIV. CHICAGO.



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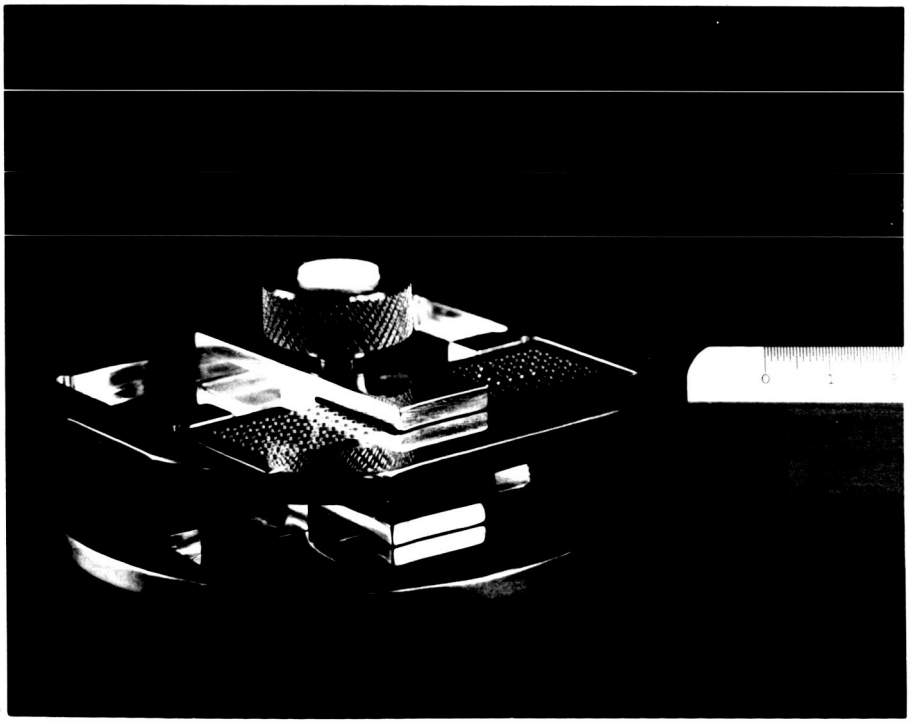

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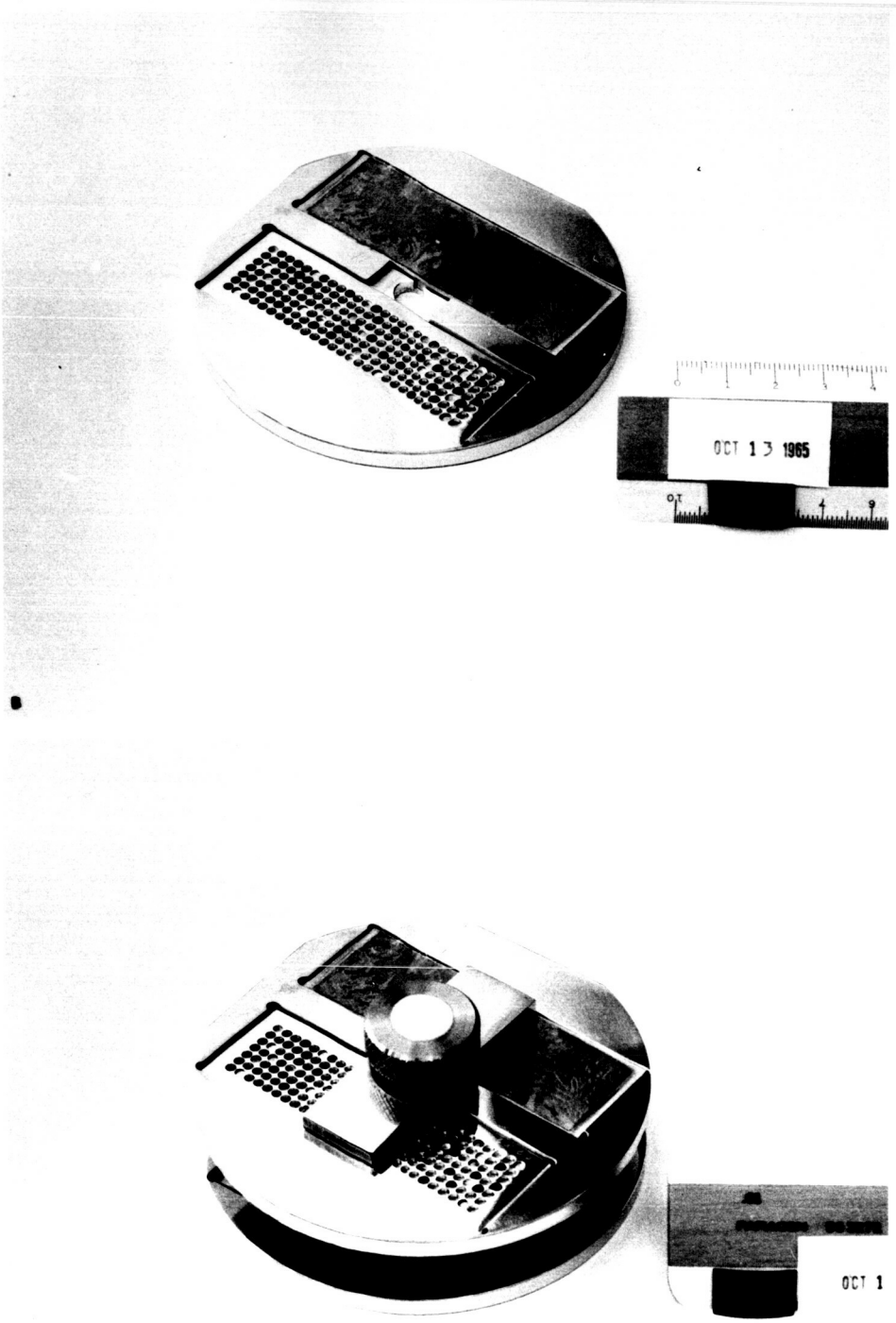
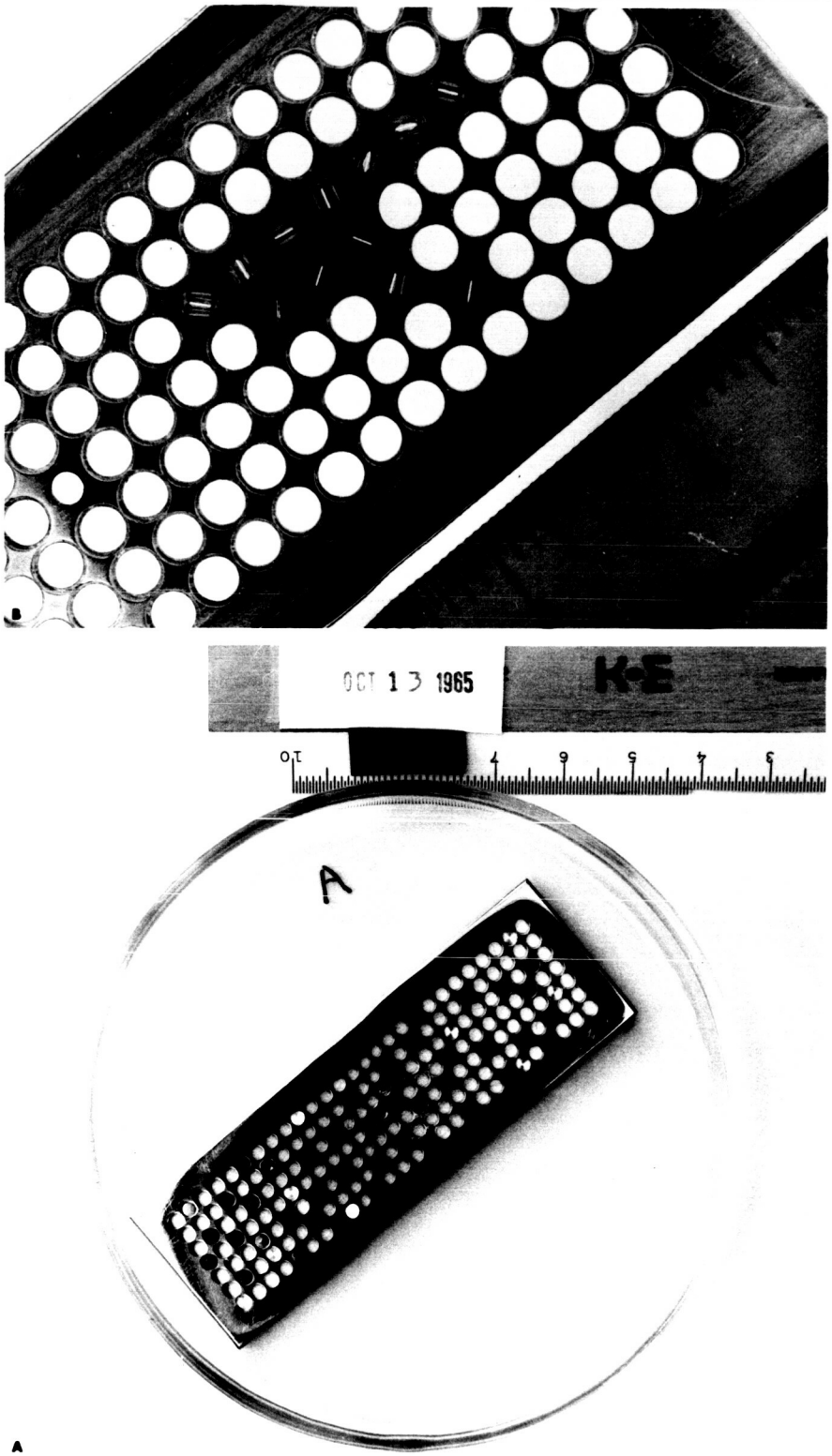
(3) FIG. 3 (a,b): HOLDERS IN SPECIAL HIGH-VACUUM CONTAINER FOR TRANSFER OF SAMPLING SURFACES TO COLLECT EXTRATERRESTRIAL MATERIAL FOR ELECTRON MICROSCOPIC, ELECTRON DIFFRACTION AND MICROPROBE ANALYSIS UNDER CONTROLLED CONDITIONS OF MINIMUM CONTAMINATION. THIS SIMPLE MODULAR DEVICE CAN BE READILY ASSEMBLED AND DISMANTLED WITH FEW MANIPULATIONS UNDER CLEAN-ROOM AND EVEN HIGH-VACUUM CONDITIONS. IT PROVIDES SECURE ATTACHMENT WITH SLIDES CONTAINING SEVERAL HUNDRED PLATINUM SPECIMEN HOLDERS COATED WITH THIN (100-400 Å) CARBON, SINGLE-CRYSTAL GRAPHITE OR MICA LAMELLAE, FORMVAR, SILICON MONOXIDE AND OTHER TYPES OF SUBSTRATES, IN ADDITION TO LARGER SURFACES OF FRESHLY-CLEAVED SINGLE-CRYSTAL MICA, PLASTIC SLIDES AND RELATED COLLECTING SURFACES SUITABLE FOR ELECTRON OPTICAL STUDIES. THREE IDENTICAL CONTAINERS AND SPECIMEN SUBSTRATE ASSEMBLIES WERE PREPARED FOR THE "LOFTER" PROJECT MICROMETEOROID SAMPLING FLIGHT SCHEDULED FOR NOVEMBER 1965 TO PROVIDE INDEPENDENT CONTAMINATION CONTROLS AT THE DIFFERENT SITES OF THE EXPERIMENT: AMES RESEARCH CENTER, ROCKET-LAUNCHING SITE, AND ACTUAL PAYLOAD SPECIMENS TO BE EXAMINED IMMEDIATELY AFTER RECOVERY AT THE ELECTRON MICROSCOPE CLEAN-ROOM LABORATORIES, DEPARTMENT OF BIOPHYSICS, UNIVERSITY OF CHICAGO.



A

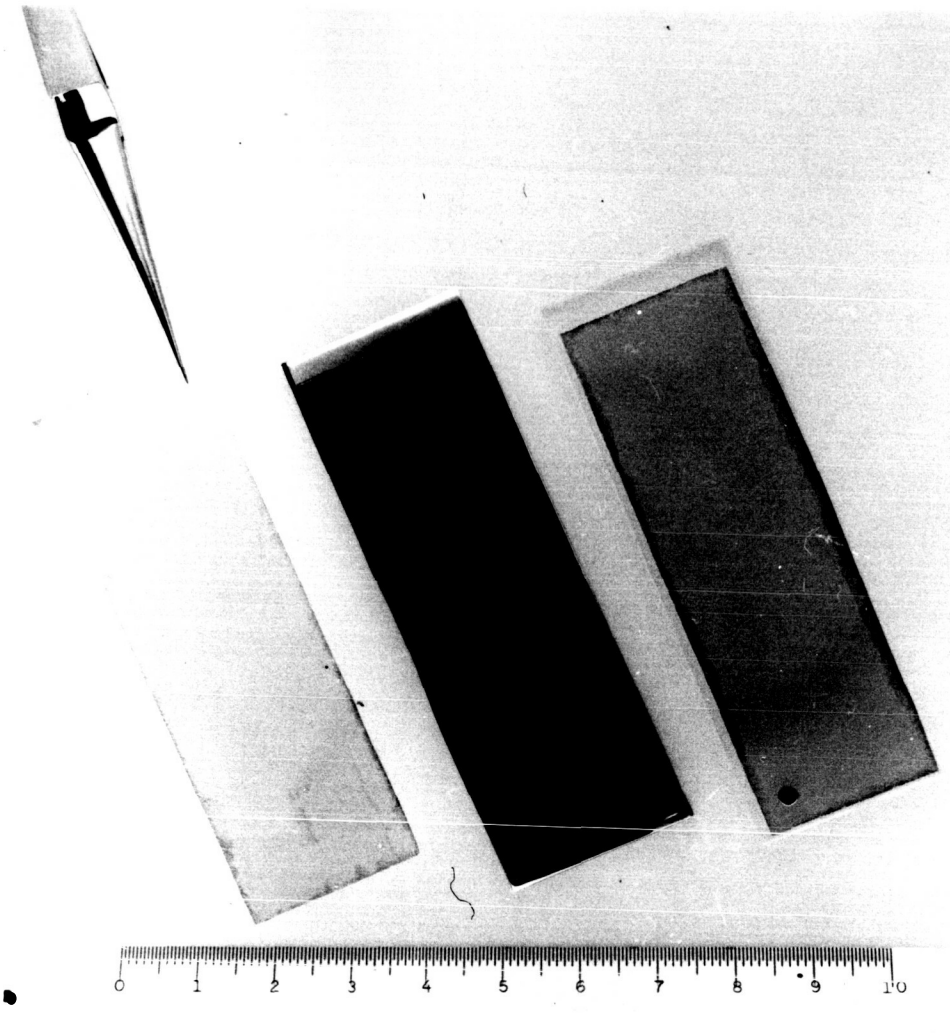
(4)

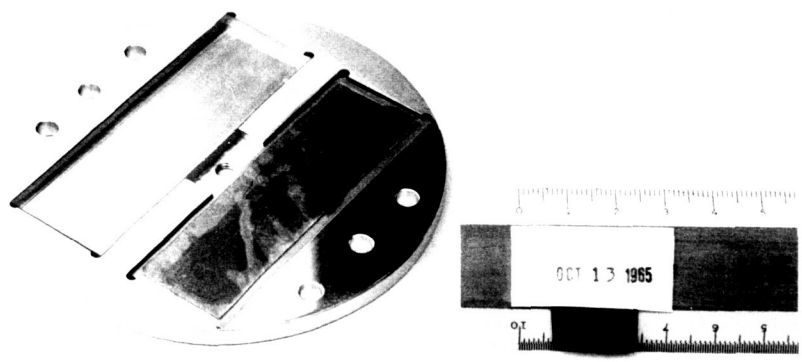
FIG. 4 (a,b): HOLDERS IN SPECIAL HIGH-VACUUM CONTAINER FOR TRANSFER OF SAMPLING SURFACES TO COLLECT EXTRATERRESTRIAL MATERIAL FOR ELECTRON MICROSCOPIC, ELECTRON DIFFRACTION AND MICROPROBE ANALYSIS UNDER CONTROLLED CONDITIONS OF MINIMUM CONTAMINATION. THIS SIMPLE MODULAR DEVICE CAN BE READILY ASSEMBLED AND DISMANTLED UNDER CLEAN-ROOM AND EVEN HIGH-VACUUM CONDITIONS FOR DIRECT TRANSFER OF SPECIMENS TO ELECTRON MICROSCOPE. IT PROVIDES SECURE ATTACHMENT FOR SLIDES CONTAINING SEVERAL HUNDRED PLATINUM SPECIMEN HOLDERS COATED WITH THIN FILM SUBSTRATES OF DIFFERENT TYPES, IN ADDITION TO LARGER SAMPLING SURFACES OF FRESHLY-CLEAVED SINGLE-CRYSTAL MICA, PLASTIC SLIDES AND RELATED COLLECTING SURFACES SUITABLE FOR ELECTRON-OPTICAL STUDIES TO BE CARRIED OUT AT THE ELECTRON MICROSCOPE CLEAN-ROOM LABORATORIES, DEPARTMENT OF BIOPHYSICS, UNIVERSITY OF CHICAGO. (Scale in mm.)



A

(5) FIG. (5.1): CELLS WITH PLATINUM SPECIMEN HOLDERS IN SPECIAL HIGH-VACUUM TRANSFER CONTAINER TO COLLECT EXTRATERRESTRIAL MATERIAL FOR ELECTRON MICROSCOPIC, ELECTRON DIFFRACTION AND RELATED ELECTRON OPTICAL STUDIES UNDER CONTROLLED CONDITIONS OF MINIMUM CONTAMINATION. THE PLATINUM SPECIMEN HOLDERS WITH SLITS AND HOLES OF DIFFERENT CONFIGURATION ARE MORE SUITABLE THAN THE STANDARD THIN COPPER MESH ELECTRON MICROSCOPE SPECIMEN HOLDERS FOR THESE CRITICAL STUDIES. THE PLATINUM HOLDERS ARE COATED WITH ULTRATHIN (100- [illegible] Å) CARBON, FORMVAR, SILICON MONOXIDE FILMS, METAL-SHADOWED GRAPHITE OR MICA LACEWORK AND OTHER TYPES OF SUBSTRATES SUITABLE FOR HIGH RESOLUTION ELECTRON MICROSCOPY AND ELECTRON DIFFRACTION. THE FILMS WERE DEPOSITED IN A HYDRO-CARBON FREE, ULTRA-HIGH VACUUM UNIT WITH VARIAN ION-PUMP (10^{-7} to 10^{-8} mm Hg.) IDENTICAL SPECIMENS WERE PREPARED FOR THE THREE CONTROL CONTAINERS AT THE DIFFERENT SITES OF THE EXPERIMENTS RELATED WITH THE "LUSTER" PROJECT SAMPLING FLIGHT SCHEDULED FOR NOVEMBER 1965. (Scale in mm).





(6)

FIG. 6 (a,b): SLIDES OF FRESHLY CLEAVED SINGLE-CRYSTAL MICA AND OF LUCITE IN SPECIAL HIGH-VACUUM TRANSFER CONTAINER TO COLLECT EXTRATERRESTRIAL MATERIAL FOR ELECTRON MICROSCOPIC, ELECTRON DIFFRACTION AND RELATED ELECTRON OPTICAL STUDIES UNDER CONTROLLED CONDITIONS OF MINIMUM CONTAMINATION. THE FRESHLY-CLEAVED SINGLE-CRYSTAL MICA SURFACES PROVIDE IDEAL, CLEAN AND ATOMICALLY SMOOTH SURFACES FOR COLLECTION AND EXAMINATION OF MICROMETEORITES AND EXTRATERRESTRIAL SPECIMENS. THE LUCITE SLIDE WOULD PROVIDE SUITABLE SUBSTRATES FOR EMBEDDING OF THE IMPACTING MICROMETEORITES WHICH COULD THEN BE SUBSEQUENTLY ULTRATHIN SECTIONED WITH A DIAMOND KNIFE AND ULTRAMICROTOME FOR ELECTRON MICROSCOPY AND ELECTRON DIFFRACTION (Fernandez-Moran, 1953-65). IDENTICAL SPECIMENS WERE PREPARED FOR THE THREE CONTROL CONTAINERS AT THE DIFFERENT SITES OF THE EXPERIMENTS RELATED TO THE "LUSTER" PROJECT SAMPLING FLIGHT SCHEDULED FOR NOVEMBER 1965. (Scale in mm.)

AVAILABLE FACILITIES:

The proposed research and training program will be carried out in the special laboratory facility for high resolution electron microscopy recently completed in the Research Institutes of the University of Chicago with funds provided by the University of Chicago (approximately $250,000), NASA Grant NSG 441-63, NIH Grant B-2460, and NIH Grant NB 04267, and AEC Contract AT 30-1-2278. (Estimated total cost of facilities: $750,000).

These laboratories occupy a total of about 4,000 square feet and comprise: 5 high resolution electron microscopes installed on individual vibration control bases, provided with a special highly regulated power supply (located in a special, separated air-conditioned enclosure). This 50-kilowatt motor generator set, specially designed and manufactured by Westinghouse Corporation is equipped with a new solid state regulator, giving better than 0.1 % voltage stability and very low harmonic distortion. The laboratory facilities also include ultrahigh vacuum Varian evaporation units, 4 ultramicrotomes, special cryogenic facilities for low-temperature ultramicrotomy, light microscopes, and complete preparation and photographic darkroom facilities. Adjoining laboratories include additional electron diffraction and x-ray diffraction facilities, one of which is provided for low-temperature x-ray diffraction. During the past year, we have completed the organization, testing and operation of the electron microscope laboratories and expanded our facilities into rooms 203B, 205, and 207 for adjacent laboratory space. Plans are now laid for renovation and construction in Room 20 in the basement of the Research Institutes. This room is to be used for the isolated microscope installation and will include a ten-ton concrete block set off from the foundation of the building by Korfund type USD vibro isolators, designed especially for this purpose by Mr. Jack Harris of Korfund. We are ready to procede with our construction of special air conditioning, with ventillation system from the adjoining central air conditioning room, preparation of the pit for the special foundation, followed by floor and acoustical installation to complete the isolation of this room from the normal dust and vibrations of the building atmosphere. Provisions will also be made for installation of the special trolley crane to be provided for lifting the heavy components of the microscope.

This research unit is probably the only existing electron microscope facility which is especially equipped to operate under "clean room" conditions. High resolution electron microscopy can be carried out under ideal conditions, independent of ambient conditions, preactically on a 24-hour basis. This stands in contrast to the vast majority of electron microscopy installations

which are constantly subject to random and environmental perturbations (line voltage fluctuations, etc.). Favorable location of the research unit close to the low-temperature facilities, the new laboratory for Computer Research and the Enrico Fermi Institute for Nuclear Studies is of key value for the contemplated research and training program. Under such favorable conditions this unit could develop gradually as the nucleus of a larger national and international research and training center for high resolution electron microscopy and molecular biology. (See accompanying Annual Progress Report).

BIOGRAPHICAL SKETCH

Humberto Fernandez-Moran, Principal Investigator

1. Title: Professor of Biophysics
2. Birth: [redacted]
3. Nationality: Venezuelan
4. Sex: Male
5. Curriculum Vitae:

1939-1940	A.B. (Matura) Schulgemeinde Wickersdorf, Saalfeld (Germany).
1944	M.D. University of Munich (Germany)
1945	M.D. University of Caracas (Venezuela)
1951	M.S. Cell Biology, University of Stockholm
1952	Ph.D. in Biophysics, University of Stockholm
1945-1946	Fellow in Neurology and Neuropathology, George Washington University, Washington, D.C., U.S.A.
1946-1948	Foreign Assistant, Neurosurgical Clinic, Serafimer-laserettet, Stockholm
1947-1949	Research Fellow, Nobel Institute of Physics, Stockholm
1948-1951	Research Fellow, Inst. for Cell Research and Genetics, Karolinska Inst., Stockholm
1951-1958	Professor and Chairman, Dept. of Biophysics, University of Caracas (Venezuela)
1952	Asst. Prof. of Biophysics, Inst. for Cell Research and Genetics, Karolinska Inst., Stockholm
1954-1958	Director, Venezuelan Inst. for Neurology and Brain Research, Caracas
1958-1962	Assoc. Biophysicist, Neurosurgical Service, Massachusetts General Hospital, Boston
1958-1962	Visiting lecturer, Dept. of Biology, Massachusetts Institute of Technology, Cambridge
1958-1962	Research assoc. in Neuropathology, Harvard University, Cambridge
1962	Prof. of Biophysics, Dept. of Biophysics, The University of Chicago, Chicago, Illinois

6. Societies
   Member XXVI, Academia Ciencias Fisicas y Matematicas, Caracas
   Honorary corresponding member, American Academy of Neurology
   Member, Committee, International Society for Cell Biology
   Member, Societies of Neurology of Buenos Aires, Santiago de Chile, Lima, Peru, S.A.
   Member, Societies of Surgery of Buenos Aires, Santiage de Chile, Lima, Porto Alegre, S.A.
   Member, Electron Microscopy Society of America
   Member, American Nuclear Society
   Section Office, Pan American Medical Association
   Member, American Academy of Arts and Sciences, 1964
7. Other Appointments:

1947-1954	Scientific and Cultural Attache to the Legations of Venezuela in Sweden, Norway, Denmark
1955	Head, Venezuelan Comm. to Atomic Energy Conference in Geneva, Switzerland.

1957 Chairman, Venezuelan Comm., 1st Inter-American Symposium on Nuclear Energy, Brookhaven, N.Y.
1958 Minister of Education of Venezuela
1958 Member, OAS Advis. Comm. on Science Development in Latin America, National Academy of Sciences
1957 Member, U.S. National Cmn., UNESCO, 1957
1961 Member, Editorial Board, Journal of Cell Biology

IV. Acknowledgements.

It is a pleasure to thank Dr. H. Stanley Bennett, Dean Division of Biological Sciences; Dr. Raymond E. Zirkle, Chairman, Department of Biophysics; and Dr. William Bloom, Department of Biophysics, University of Chicago for their valuable suggestions and whole-hearted support of our program.

We are indebted to Mr. C. L. Berrington, Mr. G. Monito, Mr. A. Peuron, Mr. D. Kasun, Dr. S. Autler, Dr. J. Hulm, and Mr. Fred Lins of the Cryogenics Systems Department of Westinghouse Electric Corporation for their valuable cooperation and supervision.

We are particularly indebted to Mr. R. Szara and Prof. L. Meyer of the Low Temperature Laboratory; Mr. John Hanacek and Mr. G. Gibson of the Machine Shop; and Mr. Akerhaugen, Mr. John Costa, and Mr. Helmut Krebs of the Central Development Shop for their most valuable suggestions and technical assistance.

We are, of course, greatly obliged to our own staff: Mr. L. Ouwerkerk, Dr. K. Yada, Dr. E.F.J. van Bruggen, Mr. R. Luftig, Mr. M. Ohtsuki, Mr. C. Hough, Mr. E. Dunker, Mr. C. Weber, Mr. H. Schilder, Miss L. LaDeur, Miss C. Runner, Miss D. Meddoff, Mrs. S. Schmidt, Miss A. Hollinger, Miss M. Arnold, Mrs. M. Devere, & Miss J. Richardson; and to our colleagues: Dr. R. Haselkorn, A. Colvill, and H. Manor for their active participation in carrying out this project.

We wish to thank Mr. G. Olson and Mr. W. Connett of the Department of Buildings and Grounds; Mr. C. Mokstad, Asst. to Dean, Division of Physical Sciences; Mr. Tracht, Division Budget Office; and Miss Irene Fagerstrom and Mr. Donald Sigal of the Office of the Vice-President for Special Projects for their kind help.

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NASA GRANT NsG 441-63
ANNUAL PROGRESS REPORT
1965-1966
H. Fernández-Morán

IV. List of Publications for 1965-1966. (25 copies of each will be sent with this report.)

A. Fernández-Morán, H. New Approaches in Correlative Studies of Biological Ultrastructure by High Resolution Electron Microscopy, paper presented at the Celebration of the Tercentenary of the Microscope in Living Biology, the Royal Microscopical Society, Bethesda, Md., April 7-9 (1963). Published in Journal of the Royal Microscopical Society, Vol. 83, Parts 1 & 2, pp. 183-195 (1964).

B. Fernández-Morán, H.; and L. J. Reed; M. Koike; and C.R. Willms, Correlated Electron Microscopic and Biochemical Studies of a Multienzyme Complex: Pyruvate Dehydrogenase Complex of Escherichia coli. Published in Science, Vol. 145, pp. 930-932, June (1964).

C. Fernández-Morán, H. Analytical Systems for Biological Study of Mars: The role of the electron microscope and electron optical techniques in Exobiology. Paper presented at Exobiology Summer study on the Biological Exploration of Mars, Stanford University, Berkeley, California, August, 1964. Announced in the Journal of Scientific Technical Aerospace Reports by the National Aeronautics and Space Administration. Reference No. SC/NsG-441. (1964).

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E. Fernández-Morán, H. Biological Systems as Formed by Water. Summation and General Discussion. Paper published in Proceedings of the New York Academy of Sciences, October 5-8, 1964.

F. Fernández-Morán, H. Electron Microscopy with High-Field Superconducting Solenoid Lenses. Published in Proceedings of the National Academy of Sciences, Vol. 53, No. 2, pp. 445-451. February (1965).

IV. List of Publications for 1965-1966 (con't.).

G. Fernández-Morán, H. Application of High-Field Superconducting Solenoid Lenses in Electron Microscopy. Paper presented at Annual Meeting of the National Academy of Sciences, April (1965). Abstract in Science , Vol. 147 (1965).

H. Fernández-Morán, H. Potential Use of Electron Microscopy for Ultraminiaturized Information Storage and Retrieval with Electron Optical Demagnification, Combined with Direct Retrieval of Recorded Microtape to Supplement Telemetry in Exobiology. Paper written to supplement previous paper written for Exobiology Study on the Biological Exploration of Mars, April (1965).

V. Reports and Publications Describing Research and Training Facilities.

A. The University of Chicago. REPORTS, Vol. 15, No. 2, Summer (1964): "Magnificent Magnification."

B. The American Medical Association. Journal of the American Medical Association, Vol. 189: 31-33, September 28, 1964, "Electron Microscope--Medicine's Research Tool of Unfulfilled Promise".

C. McGraw-Hill Yearbook of Science and Technology: Photographics Highlights Section, p. 386 Cited studies by Dr. Fernández-Morán on Pre-Cambrian Rocks of the Canadian Shield (Gunflint Chert Formation of Southern Ontario) 1965.

NASA Grant NsG 441-63
ANNUAL PROGRESS REPORT

List of Publications for 1965-1966 (15 copies of each are included with this report and renewal application)

1. H. Fernández-Morán, Biological Systems as Formed by Water. Summation and General Discussion, in Proceedings of the New York Academy of Sciences, October 5-8, 1964.

2. H. Fernández-Morán, Electron Microscopy with High-Field Superconducting Solenoid Lenses, in Proceedings of the National Academy of Sciences, Vol. 53, No. 2, pp. 445-451, February, 1965.

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4. E.F.J. van Bruggen and H. Fernández-Morán, Reassociation of Hemocyanins from Subunit Mixtures, submitted to the J. of Mol. Biol. for publication in 1966.

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